ORIGINAL RESEARCH PAPER
Volume - 14 | Issue - 02 | February - 2025 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ADVANCING PRENATAL CARE: THE ROLE OF PENTA MARKER IN THE FIRST TRIMESTER ANEUPLOIDY SCREENING

Clinical Biochemistry

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ABSTRACT

Aim- The main aim of this present study is to check the performance of five serum marker with ultrasound in first trimester for fetal aneuploidy. **Materials & Method-** We screened a cohort of 1159 pregnant women samples whose sample were collected between 11+0-13+6 weeks of gestation for aneuploidy detection. Levels of β- human chorionic gonadotropin (β-hCG) , pregnancy associated plasma protein-A (PAPP-A), and alpha-fetoprotein (AFP), placental growth factor (PLGF)and dimeric inhibin (DIA) were determined with using the fluoro-immunometric method on Auto DELFIA (Perkin Elmer, Turku, Finland). Results were converted to Multiples of the median and final risk was calculated. **Results-** Total 1159 women screened for combined first trimester screening and their final risk was calculated. Out of 1159 only 17 (1.4%) women showed the high risk for trisomy 21. Extra 3 markers PLGF, inhibin-A and AFP were performed on the same high risk women samples, a separate final risk was calculated with five markers. Out of 17 only 11(64%) women showed the high risk in penta screening. This expanded screening reduces the false positive risk up to (36%). **Conclusion-** This expanded first trimester screening test have high detection rate with less false positive rate comparatively other screening strategies during first trimester. Apart from high detection rate this screening also helps in the detection of other pregnancy related complications like- early onset of pre-eclampsia and fetal growth restriction (FGR).

KEYWORDS

First trimester screening, Maternal serum marker test, Dual marker, Penta marker, Fetal aneuploidy screening

INTRODUCTION-

Many children in the country are born with various type of abnormalities like neural tube defects, spina bifida,cleft palate, Down syndrome and other chromosomal abnormalities every day. No treatment is available to till date to treat all this type of chromosomal abnormality. Therefore ,emphasis has been given to disease prevention by prenatal screening **(Vondran et al, 1999; Alcantara et al, 2001)** (1,2). Prenatal genetic testing is the only way that can improve the possibility of having a healthy child while screening at antenatal clinics is the best way to identify couples at risk of having an affected child. By the late 1990's and early 2000's, the "triple marker" screen became the tool routinely used(3). It is also possible to perform the serum screening for chromosomal abnormalities in the second trimester. Second trimester screening is offered at 15-19 weeks of pregnancy. Detection rate for Down syndrome in second trimester is lower in comparison to combined first trimester screening (4). The most popular algorithm that is used for DS screening is the so-called first-trimester combined test, performed between 11+0 and 13+6 weeks of gestational age. This test is composed of the concentrations of pregnancy-associated plasma protein A (PAPP-A) and free β-hCG in maternal serum, the ultrasonographic nuchal translucency measurement (NT) in conjunction with maternal age. Detection rate for trisomy 21 of combined first trimester screening has been shown to 84-90%, with a false positive rate 5% (5,6,7).

Although some other marker like dimeric inhibin-A and PLGF were also incorporated into the the already exist logarithm of first trimester screening. Incorporation of both these marker not only improves detection rate for Down syndrome but also gives the idea of other pregnancy related complications like: Pre-eclampsia during first trimester. Several studies on pre-eclampsia suggest that if we start low dose of asprin decrease the incidence of pre-eclampsia upto 62% before starting of 16 weeks of gestation age(8). As newer tests are being integrated into existing screening programs, the triple test has been largely replaced. Some of the newer screens include: the quadruple test, where dimeric inhibin A is added to AFP, hCG, and uE3 markers. This test is offered to the patient between 14-19 weeks of gestation age but it can be offered up to 22 weeks of gestation age. Malone FD 2005 reported that the detection rate of trisomy 21 was nearly 83% which can be compared with first trimester screening(9).

There is a emerging and rapidly developing prenatal testing known as Non-invasive prenatal testing (NIPT), non-invasive prenatal diagnosis (NIPD), or non-invasive prenatal screening (NIPS). The last term is recommended by the American College of Medical Genetics to emphasize the screening nature of the test. This technique is first demonstrated by Dennis Lo et al. in 1997 which is based on cell free fetal DNA that circulate in the maternal blood(10). This fetal DNA is reliably detected after 7 completed weeks of gestation age to the late pregnancy. Another advantage of this test is the ability to diagnose cytogenetic microdeletion with high degree of accuracy. This is a safe, reliable and non-invasive method, which has high detection rate, high positive predictive value reduces the risk of miscarriage and allows early testing. Combined first trimester maternal screening shows the 92% detection rate for trisomy 21 with a 5% false positive rate(11). While the accuracy of NIPT results is 99.6%(12).

The cost of NIPT is little more than combined maternal screening and amniocentesis. This NIPT reduces the invasive procedure. The development of screening method that enhance the detection rate of first trimester aneuploidy while also screening for pre-eclampsia could revolutionize prenatal care. In this study we add three markers AFP,PLGF and inhibin-A into the already existed algorithm (β-hCG, PAPP-A) of prenatal screening. We compare the combined first trimester screening results with penta marker on the same patient sample. With such dual purpose approach would offer several significant advantages like- improved detection rate, early intervention for pre-eclampsia, cost-effectiveness, streamlined testing. The main aim of this study to evaluate the performance of five marker β-hCG, PAPP-A, inhibin-A, AFP, PLGF during the first trimester.

MATERIALS & METHODS-

Study Design: This is a prospective observational study which was conducted for a period between August 2024 to November 2024 at Redcliffe labs, Noida Uttarpradesh, India. A total of 1159 women were recruited during their first trimester screening for combined first trimester screening. A final risk was calculated from their combined screening. Women who showed the high risk in combined first trimester screening, extra three markers were processed from their remaining serum samples.The inclusion criteria were ultrasound-determined pregnancy before completed 13 weeks 6 days of gestation. The exclusion criteria were fetal demise and multiple gestation. A written informed consent was taken from all women for participation in this study.

Mode Of Screening: Patient demographic information such as age, date of last menstrual period, date of birth, weight, height, ethnicity, smoking status, history of diabetes mellitus, history of conception, drug history, bleeding history, events during past pregnancies, and history of folic acid supplementation were recorded in a proforma. An ultrasonographic (USG) scan was carried out to determine fetal viability, multiple pregnancy and gross fetal anomalies. Crown-rump

length (CRL), and biparietal diameter (BPD) measurements were made. Nuchal translucency (NT) measurement was carried out on women who had a fetal CRL between 45-84 mm, which equates to a range of gestation between 11 weeks 0 days to 13 weeks and 6 days.

Blood samples were collected from all patients during the period of gestation ranging between 11 weeks 0 days to 13 weeks and 6 day. Two ml of blood was collected by venipuncture using aseptic precautions in a red top vacutainer. It was allowed to clot for one hour. Serum was separated by centrifugation at 3000 rpm for 10 minutes and further analysis was done.

Methodology For Analytes-

The free β-hCG, PAPP-A, AFP, PLGF and dimeric inhibin-A analyses were performed on the Auto DELFIA Immunoassay system (PerkinElmer, Turku Finland). For each batch, kit calibrators and controls were run in duplicates.

For serum analyses, test sera for β-hCG, PAPP-A, AFP, PLGF and dimeric inhibin-A were poured into the wells. PAPP-A, AFP, PLGF, dimeric inhibin-A and free β-hCG bound to the coated wells and interacted with Europium- and Samarium-labeled monoclonal antibodies to PAPP-A,AFP, PLGF and dimeric inhibin-A and free β-hCG, respectively. After incubation and washing, an enhancement solution was added. The enhancement solution dissociated Europium (Eu) and Samarium (Sm) ions from the tracer antibody, and they were chelated into the solution. The amount of Eu and Sm label was measured by excitation with a 340 nm laser, and the emitted lights were detected at 610 and 650 nm, respectively. The intensity of the fluorescence was proportional to the concentration of the analytes in the sample. The quantification limit was 0.04 U/L for PAPP-A and 6.6 ng/mL for free β-hCG and for PLGF 5.6 pg/ml.

RESULTS-

Table 1: Demographic Details Of Eligible Patients-

Demographics	Total No. of women	Frequency
Regularity of menstrual cycles		
History of bleeding (+)	88	7.5%
Regular Cycles	915	79%
Irregular Cycle	243	21%
Folic Acid Supplements received	1147	98.9%
Gravidity		
Primigravida	360	30.9%
Multigravida	799	69.9%

Table 1 shows demographic and biochemical test results for the cohort of women attending the Antenatal Clinic. The median age was 24.0 years, and the median maternal weight was 52.8 kg. Twenty one 21% of the patients presented in 11wks+6 days, 53% presented in 12 weeks+6 days, and 26% were in the 13weeks+6 days group.

Table 2: Distribution Of Participants According To Gestation Age

Gestational Age	Frequency	Percentage
11 weeks - 11 weeks, 6 days	243	21%
12 weeks - 12 weeks, 6 days	614	53%
13 weeks - 13 weeks, 6 days	302	26%
Total	**1159**	**100.0%**

A total of 1159 women were recruited in this study for combined first trimester screening. Out of 1159 only 17 (1.4%) women (figure 1) shows the high risk for trisomy 21. Other three markers (AFP, PLGF & inhibin-A) were tested on the remaining sample of women having high in combined screening. Results of these three markers were combined with the previous first trimester screening results. A separate final risk with five markers was calculated.

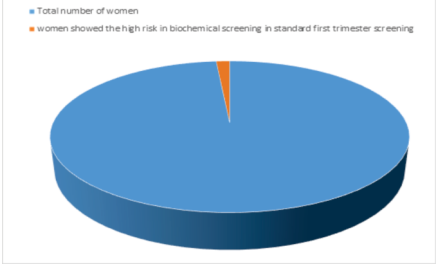

Figure 1: Total high risk women for trisomy 21 with standard combined first trimester screening-

After addition of these three markers, out of 17 only 11(64%) women showed the high risk in penta marker screening. This penta marker report neither was communicated to women nor any intervention done. Based on the combined screening results all the 17 high risk women were opted the confirmatory test amniocentesis and NIPT. Six women (36%) who showed the low risk in penta marker were showed the low risk for trisomy 21 in their confirmatory test. This penta marker screening reduces the false positive rate up to 36%.

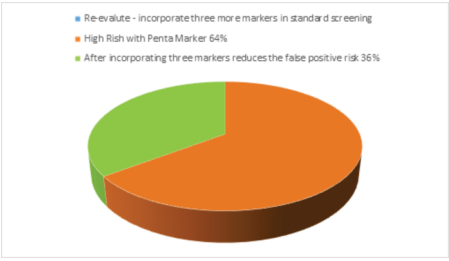

Figure 2: Total high risk women for trisomy 21 after incorporating three markers-

DISCUSSION-

As an alternative to universal cell free DNA testing, our study showed that first trimester screening performed better when additional biochemical markers (AFP, PLGF and inhibin-A) were added to the standard methodology. Our findings are consistent with earlier research that has demonstrated enhanced efficacy in extended first trimester screening when additional serum marker are included (13,14). Despite the high detection rates and low false positive rates, cell-free DNA testing is not a good choice for a main screen because to its high cost. Consequently, a number of studies have recommended the use of first trimester contingent approach.

In this manner, cell-free DNA testing is used as a follow-up for instances that surpass lower risk cut-offs (e.g., 1/1000) in this method. As an illustration of the viability of incorporating such an approach into clinical practice, a study done by Chitty et al. 2016 using data from the UK National Health Service revealed that more than 80% of patients with risks between 1/150 and 1/1000 chose to undergo NIPT after conventional screening(15).

Nevertheless, our data demonstrate enhanced efficacy for trisomy 21 detection and a lower false positive rate even with current cut-offs. According to Kaur et.al 2012 incidence of Down syndrome is very high 1 in 920 in India. So tisomy 21 has historically received greater attention from traditional aneuploidy screening than trisomy 18 and 13.(16) Carmichael Jet.al. 2017 showed the incidence of trisomy 18 and 13 can be identified with great success using traditional aneuploidy screening, some study suggest that by adding PLGF can increase efficacy(13). The main strength of this study was that we used the analytes which were already in clinically use in conjunction with ultrasonography markers. The limitation of this study was the small sample size, so we could not check the performance of trisomy 18 and 13. Another advantages, this study not only gives idea about the high risk of trisomy but also gives the idea about the pre-eclampsia in early gestation age. This expended screening would be the choice for clinician and patient because it eliminates the need for separate billing assessments and extra logistical work from doctors, this method could expedite the implementation of pre-eclampsia risk assessment.

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